EFFECT OF IRRADIANCE, SUCROSE, AND CO_2 CONCENTRATION ON THE GROWTH OF POTATO *(Solanum tuberosum L.)* in vitro

Neil C. Yorio, Dynamac Corporation, John F. Kennedy Space Center, FL

Raymond M. Wheeler, NASA Biomedical Operations and Research Office, John F. Kennedy Space Center, FL

Russell C. Weigel, Department of Biological Sciences, Florida Institute of Technology, FL

Technical Memorandum 110654

June 1995

TABLE OF CONTENTS

Abstract. Growth measurements were taken of potato plantlets *(Solanum tuberosum* L.) cvs. Norland (NL), Denali (DN), and Kennebec (KN), grown in vitro. Studies were conducted in a growth chamber, with nodal explants grown for 21 days on Murashige and Skoog salts with either 0, 1, 2, or 3% sucrose and capped with loose-fitted Magenta 2-way caps that allowed approximately 2.25 air exchanges/hour. Plantlets were exposed to either 100 or 300 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF), and the growth chamber was maintained at either 400 or 4000 μmol mol^{-1} CO_2. Regardless of PPF, all cvs. that were grown at 4000 μmol mol^{-1} CO_2, showed significant increases in total plantlet dry weight (TDW) and shoot length (SL) when sucrose was omitted from the media, indicating an autotrophic response. At 400 μmol mol^{-1} CO_2, all cvs. showed an increase in TDW and SL with increasing sucrose under both PPF levels. Within any sucrose treatment, the highest TDW for all cvs. resulted from 300 μmol m^{-2} s^{-1} PPF and 4000 μmol mol^{-1} CO_2. At 4000 μmol mol^{-1} CO_2, TDW showed no further increase with sucrose levels above 1% for cvs. NL and DN at both PPF levels, suggesting that sucrose levels greater than 1% may hinder growth when CO_2 enrichment is used.

Abbreviations: NL, Norland; DN, Denali; KN, Kennebec; PPF, photosynthetic photon flux; SL, shoot length; TDW, total dry weight; CELSS, controlled ecological life support system.

TABLES AND FIGURES

ACKNOWLEDGMENTS

This research was supported in part by funds from the Technological Research and Development Authority, Melbourne, Florida. We thank Barbara Peterson, Bill Moore, Joe Martinez (Dynamac Corporation, Kennedy Space Center, FL) and Tegi Ruffin (Morris Brown College, Atlanta, GA) for their contributions in the completion of this work. This paper is from research performed for a Masters thesis awarded by Florida Institute of Technology to Neil C. Yorio.

PRODUCT DISCLAIMER

This report, in whole or in part, may not be used to state or imply the endorsement by NASA employees of a commercial product, process, or service, or used in any other manner that might mislead.

INTRODUCTION

Recent studies involving several plant species have shown that in vitro propagation of plantlets can be accomplished autotrophically under proper environmental conditions (Cournac et al., 1991; Kozai and Iwanami, 1988). Traditionally, in vitro plantlets are grown heterotrophically on a nutrient medium containing a sugar as a carbon source (usually sucrose), under conditions of low light, high humidity, and low air exchange. By reducing or eliminating the sucrose in the media, increasing the irradiance, and allowing better air exchange or CO_2 enrichment, plantlet growth can be stimulated (Kozai et al., 1988a; Kozai and Sekimoto, 1988; Hayashi et al., 1992). Exposure to these conditions will also lead to photosynthetic competence (Dube and Vidaver, 1992; Nakayama et al., 1991), which can be comparable to that of field-grown plants (Cournac et al., 1991). Some of the obvious benefits of this approach are a reduced risk of contamination (Kozai and Iwanami, 1988), faster acclimatization, and a higher survival rate of the explants when placed ex vitro (Laforge et al., 1991).

Eliminating the use of sucrose in the media for in vitro propagation is particularly appealing when considering plant propagation in the bioregenerative or Controlled Ecological Life Support Systems (CELSS) currently under investigation by NASA (Tibbitts and Alford, 1982). In such a system, micropropagated plantlets of species such as potato could be used to plant a crop, which in turn would provide food for humans, as well as recycle CO_2 and O_2. The use of micropropagated autotrophic potato plants in a CELSS is desirable because it eliminates the use of sucrose (human food), avoids using part of the harvest to produce the next generation of plants, and minimizes the acclimatization stage the plantlets require when transferred ex vitro.

Different methods have been explored for providing more favorable growing conditions for micropropagated plants, ranging from comparisons of different vessel caps (Kozai et al., 1986 Fujiwara et al., 1989), to actively providing sterile CO_2 enriched air to each plantlet chamber (Dube and Vidaver, 1992; Nakayama et al., 1991). In our studies, we sought to compare cultural techniques that might be implemented in a CELSS plant propagation scheme and easily applied to common micropropagation practices. This approach involved testing the effects of a CO_2-enriched external atmosphere on the growth of micropropagated potatoes grown with different sucrose levels at different photosynthetic photon fluxes. However, genotypic responses to environmental parameters can vary widely in potato (Cournac et al., 1992); therefore, three cultivars of interest to CELSS were included to assess genotypic differences (Wheeler et al., 1991).

MATERIALS AND METHODS

Stock plantlet culture. White potato *(Solanum tuberosum L.),* cvs. Norland (NL), Kennebec (KN), and Denali (DN), were maintained in nodal culture (Hussey and Stacey, 1981). Micropropagation medium consisted of a modified MS media (Murashige and Skoog, 1968) containing 2% (20 g/L) sucrose, 0.7% tissue culture agar, and pH adjusted to 5.7 prior to autoclaving at 103 kPa and 121 C for 20 minutes. Plantlets were propagated with 15 ml of micropropagation medium in Pyrex 25 x 150 mm culture tubes, capped with loose-fitted Magenta 2-way caps (Carolina Biological Supply Co., Burlington, NC). Cultures were grown for 3 to 4 weeks at 23-25 C under a 16-h photoperiod and 80 to 100 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic photon flux (PPF) provided by VHO Vita-Lite flourescent lamps.

Experimental plantlet culture. Nodal explants of stock plantlets were subsequently transferred to culture tubes with 15 ml of micropropagation medium containing 0, 1, 2 or 3 % sucrose to provide 10 plantlets of each cv. at each sucrose concentration. To enhance gas exchange for the culture tube, Magenta 2-way caps were placed on the tube to allow a 1-cm gap between the top of the cap and the top of the tube. This arrangement of the culture vessel allowed increased air diffusion between the growth chamber and the culture vessel compared to fitting the cap completely against the top of the culture tube. Air exchange rates of the culture tubes were calculated to be 2.25 exchanges per hour using CO_2 as a tracer gas (Kozai et al., 1986). The cultures were placed in a reach-in growth chamber (Model M12, EGC Inc., Chagrin Falls, OH) with half of the tubes maintained at 300 μmol m^{-2} s^{-1} PPF, and the other half of the tubes maintained at 100 μmol m^{-2} s^{-1} PPF. PPF was monitored weekly at the level of the Magenta caps with a LI-190 quantum sensor (LI-COR, Lincoln, NE) with a Fluke 8060A True RMS Multimeter. Plantlets were grown for 21 days at 22 C under a 16-h photoperiod provided by VHO Vita-Lite fluorescent lamps. This configuration was repeated using CO_2 levels of 400 and 4000 μmol mol^{-1} CO_2 within the growth chamber.

Measurements. During the course of some of the experiments, CO_2 concentration within the culture vessels was periodically measured by removing a 1-ml aliquot of air from the vessel through the cap, and analyzing by gas chromatography with a thermal conductivity detector (Hewlett Packard 5880).

After 21 days, the plantlets were harvested and growth measurements were made. Measurements included shoot length (SL), number of nodes, and shoot fresh weight (SFW). Shoot dry weight (SDW) and root dry weight (RDW) were determined after the tissues were oven dried

at 70 C for 72 h. Presence and intensity of oedema (intumescence) injury were also noted visually using an arbitrary scale of 0 (not present) to 5 (severe injury).

To determine if interactions between the experimental conditions existed, a split-split plot analysis of variance (ANOVA, PC version 6.04 of SAS) was run for each cv. An observed significance level of 0.05 was used for the overall F-tests and least significant difference (LSD) comparisons.

RESULTS

Shoot length. Shoot length (SL) when averaged for all three potato cvs. showed a common trend with regard to CO_2 concentration (Figure 1): Plantlets grown at 400 μmol mol^{-1} CO_2 showed a near linear increase in SL with increasing sucrose, regardless of PPF. However, plantlets grown at 4000 μmol mol^{-1} CO_2 tended to decrease in SL with increasing sucrose greater than 1%. Within either CO_2 treatment, the SL of plantlets exposed to 100 μmol m^{-2} s^{-1} PPF was consistently longer than at 300 μmol m^{-2} s^{-1} PPF. Plantlets grown at 400 μmol mol^{-1} CO_2, regardless of PPF, were similar in SL at 3% sucrose to plantlets grown at 4000 μmol mol^{-1} CO_2. In addition, SL of plantlets grown at 400 μmol mol^{-1} CO_2 and 100 μmol m^{-2} s^{-1} PPF were similar to those grown at 4000 μmol mol^{-1} CO_2 and 300 μmol m^{-2} s^{-1} PPF at 2% sucrose. Significant three-way interactions (CO_2 x PPF x sucrose) were apparent for all cvs. (Table 1). In addition, significant two-way interactions (CO_2 x PPF and CO_2 x sucrose) and treatment main effects existed for all cvs. except for cv. DN, which showed no significant interaction between PPF and sucrose.

Number of nodes. Number of nodes produced per plantlet corresponded closely with SL for each cultivar (Table 2). With the exception of cv. NL at 300 μmol m^{-2} s^{-1} PPF, number of

nodes increased with increasing sucrose for plantlets grown at 400 μmol mol^{-1} CO_2. The number of nodes varied little between CO_2 treatments at any given sucrose or PPF level. Plantlets exposed to 4000 μmol mol^{-1} CO_2 showed a decrease in number of nodes with increasing sucrose greater than 1%, with the exceptions of cv. NL at 100 μmol m^{-2} s^{-1} PPF and cv. KN at 300 μmol m^{-2} s^{-1} PPF, which decreased with sucrose above 0%. For cv. NL number of nodes, all treatment main effects and interactions were significant, with the exception of a sucrose main effect. Only a sucrose main effect and significant interactions of CO_2 x sucrose and PPF x sucrose existed for cv. KN. For cv. DN, all treatment main effects and interactions were significant except for PPF x sucrose, which showed no significant interaction.

Dry weight accumulation. The analysis of root dry weight (RDW) revealed similar growth trends among cultivars, with the effect of CO_2 enrichment to 4000 μmol mol^{-1} CO_2 being most pronounced (Table 3). RDW of plantlets grown at 400 μmol mol^{-1} CO_2, regardless of PPF, increased as sucrose increased. In the case of cv. NL, the 100 μmol m^{-2} s^{-1} PPF treatment resulted in larger RDW than 300 μmol m^{-2} s^{-1} PPF for plantlets grown at 400 μmol mol^{-1} CO_2. For plantlets exposed to 4000 μmol mol^{-1} CO_2, RDW increased with increasing sucrose for cv. NL grown at 100 μmol m^{-2} s^{-1} PPF and cv. KN at either PPF. However, cv. NL grown at 300 μmol m^{-2} s^{-1} PPF and cv. DN at either PPF had the greatest RDW at 2% sucrose. In the 4000 μmol mol^{-1} CO_2 treatment, 300 μmol m^{-2} s^{-1} PPF resulted in the greatest RDW for all cvs. The greatest increase in RDW as a result of enriching CO_2 to 4000 μmol mol^{-1} was seen at 0% sucrose and 300 μmol m^{-2} s^{-1} PPF for cv. NL, which produced a 6-fold increase in RDW, and cv. DN, which produced a 15-fold increase in RDW. The greatest increase in RDW for cv. KN in response to CO_2 enrichment occurred at 0% sucrose and 100 μmol m^{-2} s^{-1} PPF (a 10-fold increase). The magnitude of increase in RDW with

4000 μmol mol^{-1} CO_2 enrichment decreased with increasing sucrose. For each cultivar, all treatment main effects and interactions were significant for RDW.

Shoot dry weight (SDW) analysis showed trends similar to RDW with regard to the effect of 4000 μmol mol^{-1} CO_2 enrichment (Table 4). For all cvs. grown at 400 μmol mol^{-1} CO_2, SDW increased with increasing sucrose, regardless of PPF. Similar to RDW, cv. NL had higher SDW at 100 μmol m^{-2} s^{-1} PPF than at 300 μmol m^{-2} s^{-1} PPF. For plantlets exposed to 4000 μmol mol^{-1} CO_2, SDW was greatest at 1% sucrose for cv. NL grown at both PPF levels and cv. KN grown at 100 μmol m^{-2} s^{-1} PPF. At 4000 μmol mol^{-1} CO_2, cv. DN had greatest SDW at 2% sucrose with both PPF treatments, while cv. KN continued to increase with increasing sucrose at 300 μmol m^{-2} s^{-1} PPF. The greatest increase in SDW as a result of enrichment to 4000 μmol mol^{-1} CO_2 was seen at 0% sucrose and 300 μmol m^{-2} s^{-1} PPF for all cvs., which produced a near 5-fold increase for cv. DN. The relative increase in SDW from CO_2 enrichment decreased with increasing sucrose at either PPF. For each cv., all treatment main effects and interactions were significant for SDW with the exception of cv. NL, in which the PPF x sucrose and CO_2 x PPF x sucrose interactions were not significant.

The growth of potato plantlets in terms of total dry weight (TDW) accumulation when averaged for all cvs. closely resembled trends of SL (Figure 2). Regardless of PPF, plantlets grown at 400 μmol mol^{-1} CO_2 showed a proportional increase in TDW with increasing sucrose. When the plantlets were grown at 4000 μmol mol^{-1} CO_2, an increase in growth occurred between 0 and 1% sucrose followed by no additional increase in TDW with additional sucrose.

Oedema. Depending on the environmental treatments, oedema injury developed on cvs. DN and KN plantlets, but no oedema was noted for cv. NL, regardless of treatments (Figure 3). Though cv. KN showed the most severe injury, the severity of oedema injury for both cvs. DN

and KN increased with increasing sucrose. It was apparent that plantlets grown at 300 μmol m^{-2} s^{-1} PPF showed greater incidence of injury than those grown at 100 μmol m^{-2} s^{-1} PPF, regardless of CO_2 treatment. In addition, plantlets grown at 4000 μmol mol^{-1} CO_2 also had a greater incidence of oedema than those grown at 400 μmol mol^{-1} CO_2, regardless of PPF. The combination of the high light and high CO_2 resulted in the greatest oedema injury.

CO_2 exchange. Although measurements were variable depending upon plantlet size, gas samples taken from inside the tubes showed that plantlets were capable of CO_2 fixation in the light and respiration in the dark, regardless of sucrose concentrations in the media (data not shown). When measured in the dark, plantlets grown at 300 μmol m^{-2} s^{-1} PPF and 400 μmol mol^{-1} CO_2 typically produced a slight increase in CO_2 accumulation within the vessels with increasing sucrose, while the plantlets grown at 100 μmol m^{-2} s^{-1} PPF had a much larger CO_2 accumulation. Plantlets exposed to 4000 μmol mol^{-1} CO_2 had proportionally smaller CO_2 drawdowns in the light with increasing sucrose than plantlets exposed to 400 μmol mol^{-1} CO_2. Higher irradiance resulted in a larger difference in CO_2 between the culture tubes and the growth chamber when plantlets were measured several hours into the photoperiod.

DISCUSSION

With all three cvs. averaged together, plantlets grown at 400 μmol mol^{-1} CO_2 showed an increase in SL with increasing sucrose (Figure 1), which was expected (Langford and Wainwright, 1987; Cournac et al., 1991). Higher PPF produced shorter plantlets, which is consistent with other experiments involving in vitro potato (Kozai et al., 1992). Because there was growth with 0% sucrose, and this growth was substantially increased at 4000 μmol mol^{-1} CO_2, it was evident that the

potato plantlets were capable of autotrophic growth given the proper environmental conditions. This result combined with the increased growth observed with the addition of sucrose in the media indicates a mixotrophic response. Measurements of lower CO_2 concentrations during the light period within the culture vessels also support this conclusion (data not shown). These results are consistent with similar experiments using potato (Cournac et al., 1991) and tobacco (Mousseau, 1986). However, SL has not been previously reported as a function of sucrose concentration for in vitro potatoes. This is an important consideration in terms of micropropagation where SL and internode length are factors in producing uniform plantlets.

SL was only increased with lower concentrations of sucrose when CO_2 enrichment was used. The decrease in SL when plantlets were exposed to sucrose levels greater than 1% and 4000 μmol mol^{-1} CO_2 is consistent with the decrease in growth noted at sucrose levels greater than 1% for carnation grown at 1000-1500 μmol mol^{-1} CO_2 (Kozai and Iwanami, 1988). It is noteworthy that within each PPF treatment, the SL of plantlets grown on 3% sucrose media was not different between 400 and 4000 μmol mol^{-1} CO_2, indicating a threshold of 3% sucrose, above which the benefit of CO_2 enrichment is no longer realized.

Because there was no branching during the 21-day experiments, the number of nodes is indicative of the number of leaves produced by the plantlets (Table 3). Number of nodes closely followed the SL trend, suggesting that it is dependent on SL. In general, there appeared to be no difference in number of nodes produced in vitro between sucrose concentrations within any CO_2 and PPF treatment. This result is consistent with carnation grown under ambient CO_2 conditions (Kozai et al., 1988b), carnation grown at 1000-1500 μmol mol^{-1} CO_2 (Kozai and Iwanami, 1988), and potato grown at 450-3000 μmol mol^{-1} CO_2 (Kozai et al., 1988a). Suggesting that the decrease

in SL for plantlets grown at 4000 μmol mol^{-1} CO_2 with respect to high sucrose concentrations is primarily a result of decreased internode length.

The TDW combined for all cvs. showed a response trend similar to that seen with SL and number of nodes (Figure 2). The increase in TDW with increasing sucrose at 400 μmol mol^{-1} CO_2 was due to the mixotrophic nature of the plantlets under these conditions. The stimulation of growth with 4000 μmol mol^{-1} CO_2 and 0% sucrose media was indicative of the plantlets becoming fully autotrophic under these in vitro conditions (Kozai et al., 1988a). A smaller increase in TDW with 1% sucrose was evident, followed by no further increase in growth with additional sucrose. Similar results have been described with elevated CO_2 for potato, where maximum growth (FW) occurred with 1.5% sucrose (Kozai et al., 1988a; Fujiwara et al., 1992), and for carnation with maximum growth at 1% sucrose (Kozai and Iwanami, 1988). No differences in FW of carnation were reported between 0 and 2% sucrose at any given light intensity (Kozai et al., 1990), which supports the hypothesis that only small amounts of sucrose in the media are beneficial when CO_2 enrichment is used, while larger amounts of sucrose may hinder growth.

On the basis of carbon exchange, additional sucrose in the medium beyond an optimal level may result in decreased photosynthesis. It has been shown that sucrose concentrations above 1% result in lower CO_2 uptake for rose plantlets (Langford and Wainright, 1987). Moreover, the amount of sucrose taken up by photosynthetically active carnation plantlets was highest with 1% sucrose in the medium (Kozai and Iwanami, 1988). It has been suggested that the additional sucrose in the medium may reduce Rubisco (CO_2-fixing enzyme) activity, resulting in low photosynthetic rates (Grout and Donkin, 1987). In addition, experiments involving in vitro potato in aerated vessels containing 1.5% sucrose resulted in increased production of starch (Cournac et

al., 1991), which at high concentrations may cause a feedback inhibition of photosynthesis (Delucia et al., 1985).

The variation in SDW and RDW observed for cvs. KN and DN may have been related to the high incidence of oedema injury observed (Figure 3). The oedema injury noted with cvs. KN and DN was identified by a white or pale-green callus-like formation on the upper leaves and petioles of the plantlets and has been described earlier for the same cvs. of potato grown in vitro (Wilson et al., 1993). Conditions similar to that of in vitro environments (high humidity and lack of UV-B irradiation) have been implicated in the development of oedema for tomato (Lang and Tibbitts, 1983). The fact that the oedema increased with increasing levels of sucrose, higher irradiance, and CO_2-enriched conditions suggests that the factors that generally increase growth also increase oedema. The greater dry weight for KN plantlets at 3% sucrose suggests that the hypertrophic growth of the intumescences may offset the inhibition in weight gain observed for cv. NL plantlets at sucrose levels above 1%.

CONCLUSION

The results of this study demonstrate that potato plants grown in vitro can be strongly influenced by CO_2 concentration, light intensity, and sucrose concentration of the medium. Plantlets were capable of autotrophic growth, which was enhanced with CO_2 enrichment of 4000 $\mu mol\ mol^{-1}$ external to the culture tubes. Sucrose levels in excess of 1% for potatoes grown in vitro resulted in no further growth enhancement when CO_2 enrichment is used. Plantlets produced under CO_2-enriched conditions were more vigorous, uniform, and more likely to acclimate sooner than plantlets grown under traditional in vitro conditions, although the latter remains to be tested

for these cultivars. The findings are important when considering in vitro propagation of potatoes in a CELSS, where sucrose may be a costly commodity to provide, but CO_2 enrichment may be easily implemented.

Table 1. Shoot length of potato cultivars tested with regard to $[CO_2]^z$, sucrose, and PPF.

PPF (µmol m^{-2} s^{-1})	S (%)	Norland $[CO_2]$ µmol mol^{-1} 400	Norland $[CO_2]$ µmol mol^{-1} 4000	Kennebec $[CO_2]$ µmol mol^{-1} 400	Kennebec $[CO_2]$ µmol mol^{-1} 4000	Denali $[CO_2]$ µmol mol^{-1} 400	Denali $[CO_2]$ µmol mol^{-1} 4000
		cm/plant		cm/plant		cm/plant	
100	0	4.2	10.3	5.0	10.1	4.2	11.6
	1	4.7	9.8	6.2	10.9	6.1	12.1
	2	5.5	8.7	7.5	9.3	7.2	11.3
	3	6.0	7.1	9.2	6.4	7.9	10.1
300	0	3.1	7.4	3.1	8.3	2.3	6.6
	1	3.4	8.8	3.7	7.6	3.5	9.0
	2	3.1	6.2	4.1	5.6	4.5	7.7
	3	2.9	4.1	4.6	5.2	5.4	6.5

CO_2	***	***	***
PPF	***	***	***
CO_2 x PPF	**	***	***
SUCROSE	***	***	***
CO_2 x SUCROSE	***	***	***
PPF x SUCROSE	***	***	NS
CO_2 x PPF x SUCROSE	***	***	***

NS,*,**,*** Nonsignificant or significant at P = 0.05, P = 0.01, and P = 0.001, respectively.
$^z[CO_2]$ maintained external to culture tubes.

Table 2. Number of nodes of potato cultivars tested with regard to $[CO_2]^z$, sucrose, and PPF.

PPF (μmol m^{-2} s^{-1})	S (%)	Norland $[CO_2]$ $\mu mol\ mol^{-1}$ 400	Norland $[CO_2]$ $\mu mol\ mol^{-1}$ 4000	Kennebec $[CO_2]$ $\mu mol\ mol^{-1}$ 400	Kennebec $[CO_2]$ $\mu mol\ mol^{-1}$ 4000	Denali $[CO_2]$ $\mu mol\ mol^{-1}$ 400	Denali $[CO_2]$ $\mu mol\ mol^{-1}$ 4000
		#/plant		#/plant		#/plant	
100	0	7.5	10.1	7.2	7.6	6.8	7.4
	1	7.8	9.6	7.8	8.1	7.4	7.5
	2	7.9	9.8	8.0	7.9	7.9	7.4
	3	8.4	9.6	8.6	7.8	7.9	7.6
300	0	7.9	10.5	7.7	8.8	6.5	8.4
	1	7.9	10.7	7.9	8.4	6.9	8.4
	2	7.9	9.9	7.8	8.0	7.1	9.0
	3	7.8	9.4	8.0	8.0	7.6	8.6

	Norland	Kennebec	Denali
CO_2	***	NS	***
PPF	*	NS	***
CO_2 x PPF	*	NS	***
SUCROSE	NS	*	***
CO_2 x SUCROSE	***	***	***
PPF x SUCROSE	***	***	NS
CO_2 x PPF x SUCROSE	*	NS	***

NS,*,**,*** Nonsignificant or significant at P = 0.05, P = 0.01, and P = 0.001, respectively.
$^z[CO_2]$ maintained external to culture tubes.

Table 3. Root dry weight of potato cultivars tested with regard to $[CO_2]^r$, sucrose, and PPF.

PPF (μmol m^{-2} s^{-1})	S (%)	Norland $[CO_2]$ $\mu mol\ mol^{-1}$		Kennebec $[CO_2]$ $\mu mol\ mol^{-1}$		Denali $[CO_2]$ $\mu mol\ mol^{-1}$	
		400	4000	400	4000	400	4000
		mg/plant		mg/plant		mg/plant	
100	0	3.3	9.4	1.0	10.6	1.1	6.9
	1	3.9	13.9	3.4	18.9	3.1	10.3
	2	7.7	14.5	7.5	20.1	4.9	13.2
	3	9.3	17.3	8.6	21.2	5.8	11.4
300	0	2.3	14.8	2.3	18.3	0.7	10.8
	1	4.0	19.0	7.2	29.6	4.4	18.3
	2	5.4	23.9	9.6	33.3	7.9	22.8
	3	8.8	18.1	10.6	47.6	10.7	16.8

CO_2	***	***	***
PPF	***	***	***
CO_2 x PPF	***	***	***
SUCROSE	***	***	***
CO_2 x SUCROSE	***	***	***
PPF x SUCROSE	***	***	***
CO_2 x PPF x SUCROSE	***	***	***

NS,*,**,*** Nonsignificant or significant at P = 0.05, P = 0.01, and P = 0.001, respectively.
$^r[CO_2]$ maintained external to culture tubes.

Table 4. Shoot dry weight of potato cultivars tested with regard to $[CO_2]'$, sucrose, and PPF.

PPF ($\mu mol\ m^{-2}\ s^{-1}$)	S (%)	Norland $[CO_2]$ $\mu mol\ mol^{-1}$ 400	Norland 4000	Kennebec $[CO_2]$ $\mu mol\ mol^{-1}$ 400	Kennebec 4000	Denali $[CO_2]$ $\mu mol\ mol^{-1}$ 400	Denali 4000
		mg/plant		mg/plant		mg/plant	
100	0	11.4	29.9	8.1	39.0	6.9	34.8
	1	14.7	37.6	14.1	56.2	11.3	46.2
	2	16.9	33.7	19.5	53.6	15.6	50.5
	3	20.0	29.3	24.7	51.6	17.9	48.1
300	0	8.0	37.2	10.1	54.1	7.2	50.7
	1	12.9	47.3	12.8	65.5	12.8	82.6
	2	14.9	44.9	22.2	73.7	22.8	87.8
	3	18.7	36.9	34.6	86.8	26.5	72.7

CO_2	***	***	***
PPF	***	***	***
CO_2 x PPF	***	***	***
SUCROSE	***	***	***
CO_2 x SUCROSE	***	***	***
PPF x SUCROSE	NS	***	***
CO_2 x PPF x SUCROSE	NS	***	***

NS,*,**,*** Nonsignificant or significant at P = 0.05, P = 0.01, and P = 0.001, respectively.
'$[CO_2]$ maintained external to culture tubes.

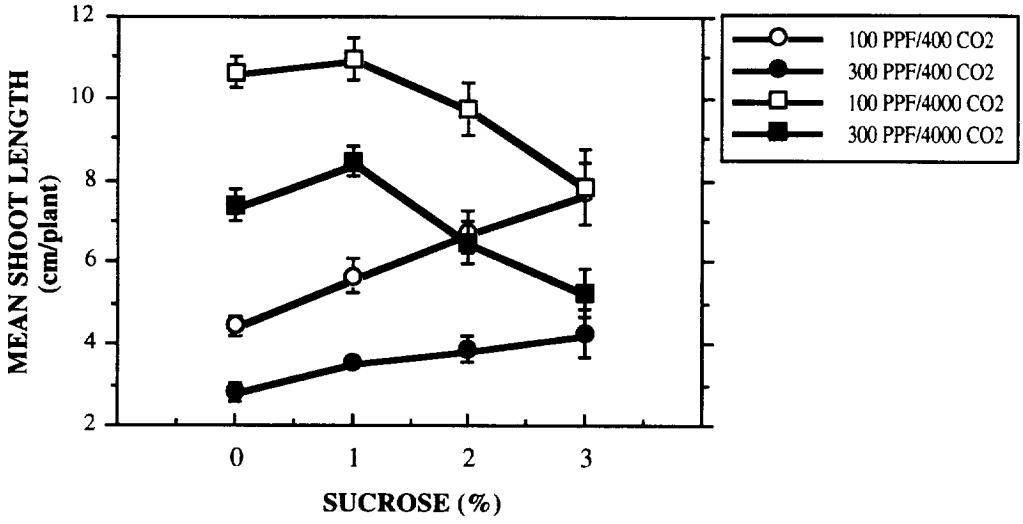

Figure 1. Combined mean shoot length for potato cvs. Norland, Denali, and Kennebec grown in vitro with regard to sucrose concentration. Vertical bars = SE of the combined means of each cv.

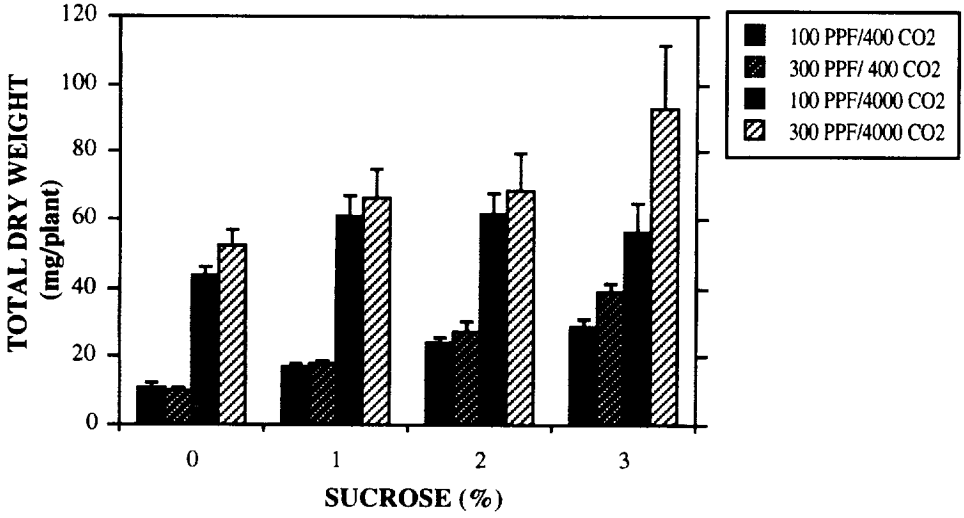

Figure 2. Combined mean total dry weight for potato cvs. Norland, Denali, and Kennebec grown in vitro with regard to sucrose concentration. Vertical bars = SE of the combined means of each cv.

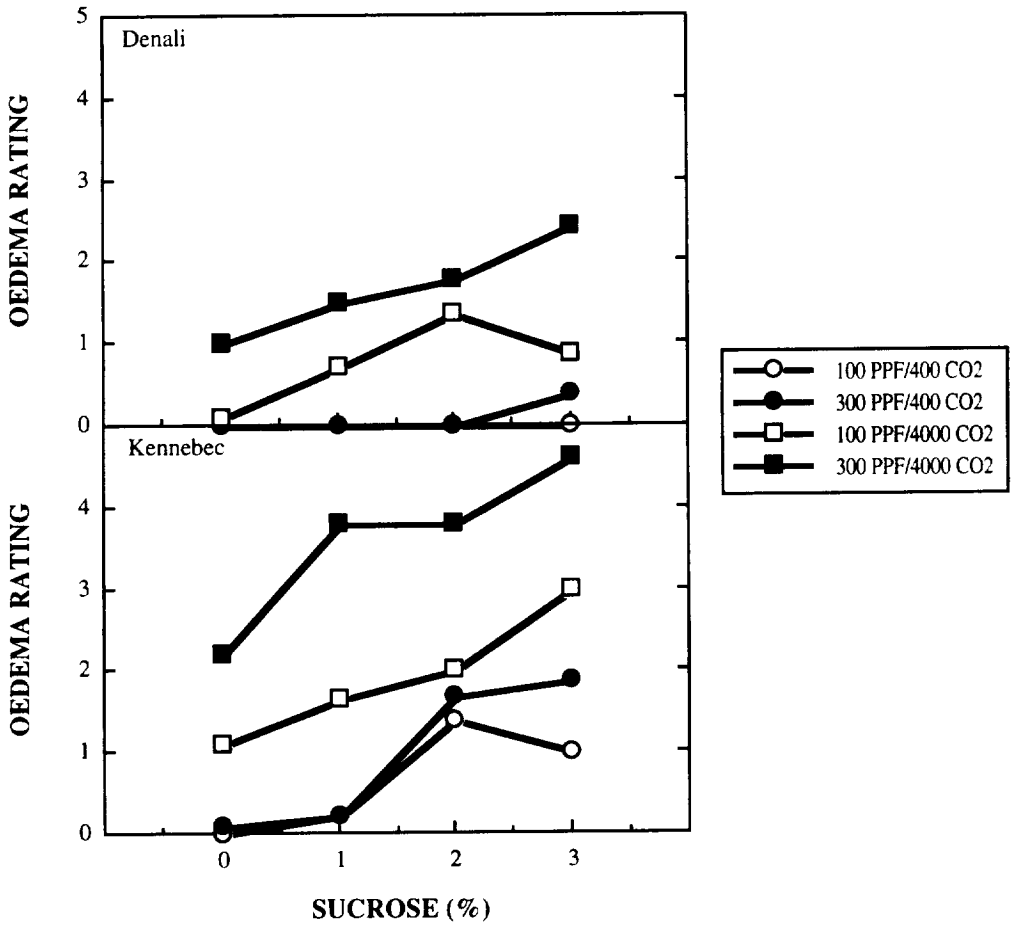

Figure 3. Oedema occurrence with potato cvs. Denali,and Kennebec grown in vitro. Ratings made visually based on a scale of 0 (no presence) to 5 (severe injury).

REFERENCES

Cournac, L., B. Dimon, P. Carrier, A. Lohou and P. Chagvardieff. 1991. Growth and photosynthetic characteristics of *Solanum tuberosum* plantlets cultivated *in vitro* in different conditions of aeration, sucrose supply, and CO_2 enrichment. Plant Physiol. 97:112-117.

Cournac, L., I. Cirier and P. Chagvardieff. 1992. Improvement of photoautotrophic *Solanum tuberosum* plantlet culture by light and CO_2: Differential development of photosynthetic and varietal constraints. Acta Hort. 319:53-58.

Delucia, E.H., T.W. Sasek, and B.R. Strain. 1985. Photosynthetic inhibition after long-term exposure to elevated levels of atmospheric carbon dioxide. Photosynthesis Res. 7:175-184.

Dube, S.L. and W. Vidaver. 1992. Photosynthetic competence of plantlets grown in vitro. A automated system for measurement of photosynthesis in vitro. Physiol. Plant. 84:409-416.

Fujiwara, K., T. Kozai, Y. Nakajo, and I. Watanabe. 1989. Effects of closures and vessels on light intensities in plant tissue culture vessels. J. Agr. Meteorol. 45:143-149.

Fujiwara, K., S. Kira, and T. Kozai. 1992. Time course of CO_2 exchange of potato cultures *in vitro* with different sucrose concentrations in the culture medium. J. Agr. Meteorol. 48:49-56.

Grout, B.W.W. and M.E. Donkin. 1987. Photosynthetic activity of cauliflower meristem culture in vitro and at transplanting into soil. Acta Hort. 212:323-327.

Hayashi, M., N. Fujita, Y. Kitaya, and T. Kozai. 1992. Effect of sideward lighting on the growth of potato plantlets *in vitro.* Acta Hort. 319:163-166.

Hussey, G. and M.J. Stacey. 1981. *In vitro* propagation of potato *(Solanum tuberosum* L.). Ann. Bot. 787-796.

Kozai, T., Y. Koyama, and I. Watanabe. 1988a. Multiplication and rooting of potato plantlets in vitro with sugar-free medium under high photosynthetic photon flux. Acta Hort. 230:121-127.

Kozai, T., C. Kubota, and I. Watanabe. 1988b. Effects of basal medium composition on the growth of carnation plantlets in auto- and mixotrophic tissue culture. Acta. Hort. 230:159-166.

Kozai, T. and K. Sekimoto. 1988. Effects of the number of air exchanges per hour of the closed vessel and the photosynthetic photon flux on the carbon dioxide concentration inside the vessel and the growth of strawberry plantlets *in vitro.* Environ. Control Biol. 26:21-29.

Kozai, T. and Y. Iwanami. 1988. Effects Of CO_2 enrichment and sucrose concentration under high photon flux on plantlet growth of carnation *(Dianthus caryophyllus* L.) in tissue culture during the preparation stage. J. Jap. Soc. Hort. Sci. 57:279-288.

Kozai, T., K. Fujiwara, and I. Watanabe. 1986. Fundamental studies of environments in plant tissue culture vessels. (2) Effects of stoppers and vessels on gas exchange rates between inside and outside of vessels closed with stoppers. J. Agr. Meteorol. 42:119-127.

Kozai, T., C. Kubota, and I. Watanabe. 1990. The growth of carnation plantlets *in vitro* cultured photoautotrophically and photomixotrophically on different media. Environ. Control Biol. 28:21-27.

Kozai, T., S. Kushihashi, C. Kubota, and K. Fujiwara. 1992. Effect of the difference between photoperiod and dark period temperatures, and photosynthetic photon flux density on the shoot length and growth of potato plantlets in vitro. J. Jap. Soc. Hort. Sci. 61:93-98.

Laforge, F., C. Lussier, Y. Desjardins, and A. Gosselin. 1991. Effect of light intensity and CO_2 enrichment during in vitro rooting on subsequent growth of plantlets of strawberry, rasberry and and asparagus in acclimatization. Scientia. Hort. 47:259-269.

Lang, S.P. and T.W. Tibbitts. 1983. Factors controlling intumescence development on tomato plants. J. Amer. Soc. Hort. Sci. 108:93-98.

Langford, P.J. and H. Wainwright. 1987. Effects of sucrose concentrations on the photosynthetic ability of rose shoots *in vitro.* Ann. Bot. 60:633-640.

Mousseau, M. 1986. CO_2 enrichment in vitro. Effect on autotrophic and heterotrophic cultures of *Nicotiana tabacum (var. Samsun).* Photosythesis Res. 8:187-191.

Murashige, T. and F. Skoog. 1968. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Nakayama, M., T. Kozai, and K. Watanabe. 1991. Effect of the presence/absence of sugar in the medium and natural/forced ventilation on the net photosynthetic rates of potato explants *in vitro.* Plant Tissue Cult. Lett. 8:105-109.

Tibbitts, T.W. and D.K. Alford. 1982. Controlled environment life support system: Use of higher plants. Conference Publication 2231, NASA Sci. Tech. Inf. Br., Moffet Field, CA. pp. 1-26.

Wheeler, R.M., T.W. Tibbitts, and A.H. Fitzpatrick. 1991. Carbon dioxide effects on potato growth under different photoperiods and irradiance. Crop Sci. 31:1209-1213.

Wilson, D.A., R.C. Weigel, R.M. Wheeler, and J.C. Sager. 1993. Light spectral quality effects on the growth of potato *(Solanum tuberosum* L.) nodal cuttings in vitro. In Vitro Cell. Dev. Biol. P:5-8.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED

4. TITLE AND SUBTITLE

Effect of Irradiance, Sucrose, and CO_2 Concentration on the Growth of Potato (Solanum tuberosum L.) in vitro

5. FUNDING NUMBERS

NAS10-12180

6. AUTHOR(S)

Neil C. Yorio, M.S., Dynamac Corporation; Raymond M. Wheeler, Ph.D., NASA; Russell C. Weigel, Ph.D., Florida Institute of Technology

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Dynamac Corporation, Mail Code DYN-3 (NCY) and NASA Biomedical Operations and Research Office, Mail Code MD-RES (RMW), Kennedy Space Center, Florida 32899; Florida Institute of Technology (RCW), Melbourne, Florida 32901

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Unclassified - Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Growth measurements were taken of potato plantlets (Solanum tuberosum L.) cvs. Norland (NL), Denali (DN), and Kennebec (KN), grown in vitro. Studies were conducted in a growth chamber, with nodal explants grown for 21 days on Murashige and Skoog salts with either 0, 1, 2, or 3% sucrose and capped with loose-fitted Magenta 2-way caps that allowed approximately 2.25 air exchanges/hour. Plantlets were exposed to either 100 or 300 umol m^{-2} s^{-1} photosynthetic photon flux (PPF), and the growth chamber was maintained at either 400 or 4000 umol mol^{-1} CO_2. Regardless of PPF, all cvs. that were grown at 4000 umol mol^{-1} CO_2 showed significant increases in total plantlet dry weight (TDW) and shoot length (SL) when sucrose was omitted from the media, indicating an autotrophic response. At 400 umol mol^{-1} CO_2, all cvs. showed an increase in TDW and SL with increasing sucrose under both PPF levels. Within any sucrose treatment, the highest TDW for all cvs. resulted from 300 umol m^{-2} s^{-1} PPF and 4000 umol mol^{-1} CO_2. At 4000 umol mol^{-1} CO_2, TDW showed no further increase with sucrose levels above 1% for cvs. NL and DN at both PPF levels, suggesting that sucrose levels greater than 1% may hinder growth when CO_2 enrichment is used.

14. SUBJECT TERMS

potato, Solanum tuberosum L., in vitro growth, irradiance, sucrose, CO_2-enrichment

15. NUMBER OF PAGES

26

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited